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ROLE OF *GLOMUS MOSSEAE* AND NEEMEX IN THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON TOMATO

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ABSTRACT

Root-knot nematodes are one of the major limiting factors for vegetables including tomato. Chemical control of nematodes is harmful to health and causes environmental pollution by contaminating underground water. Use of biocontrol agents can be the potential alternative to chemicals. Therefore, in the present study, effectiveness of mycorhizal fungus (MF) *Glomus mosseae* and neemex alone and in combination was evaluated against *Meloidogyne incognita* under greenhouse conditions. The combined application of MF and neemex caused significant reductions in number of galls, egg masses and females followed by application of MF and neemex alone as compared to control. Number of juveniles (J2)/root system and J2/100 cm^3 soil were estimated to be lower in the combined treatment of MF and neemex, followed by MF and neemex alone as compared to check. Root weight was significantly higher in *M. incognita* inoculated plants followed by MF and neemex combined treatment and MF alone in the presence of *M. incognita* as compared to other treatments. Significantly higher root length was recorded in plants treated with MF as compared to control. Shoot weight and shoot length was significantly higher in mycorrhizal treated plants as compared to other treatments. It is therefore, concluded from the present study that mycorrhizal fungus *Glomus mosseae* and neemex alone and in combination has the potential to reduce root-knot infestation and enhance growth of tomato.

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INTRODUCTION

Root-knot nematodes are serious and economically the most important pests of many cultivated crops around the world (Oka et al., 2000) and can cause the complete failure of crops. The ability of infected roots to absorb water, minerals and fertilizers is greatly hampered due to nematode attack. Serious damage of root-knot nematode, *Meloidogyne incognita*, has been recorded on tomato and other vegetables in Pakistan (Hussain et al., 2016; Kayani et al., 2013; Mukhtar et al., 2013). Crop losses become aggravated when root-knot nematodes were found in associations with other soil borne pathogens (Shahbaz et al., 2015). Use of chemical nematicides is being discouraged and gradually prohibited due to health

hazards. This can be done by adopting innocuous management practices (Martin, 2003).

Biological control of nematodes using rhizosphere micro-organisms has been advocated in several reviews to be a potential management tactic and an effective alternative to nematicides (Kerry, 2000). Mycorrhizal fungi perform a significant role as bio-protectant against pathogens (Naher et al., 2013). Mycorrhizal fungi and root-knot nematodes share a striking feature, which is their ability to form associations with the roots of the majority of plant species, whereas other biotrophs generally show a restricted host range (Trudgill and Blok, 2001). Mycorrhizal fungi and neem products have the ability to suppress root-knot nematodes in different crops including vegetables (Hasan and Khan, 2004). The role of mycorrhizal fungi in reducing harmful effects of root infection by many parasitic nematodes in crops is well recognized (Shreenivasa et al., 2007). Similarly, juveniles of *M*. *incognita* exposed to neem products show immobility and mortality (Javed et al., 2007). Neem products, particularly seed and neem cake are widely used as a soil amendment against nematodes (Akhtar and Mahmood, 1994). Neem products are absorbed by the plants and when nematodes come in contact for feeding they inhibit or delay their development (Javed et al., 2007) but no information is available on the combined application of neemex and mycorrhizal fungus for root-knot nematode management and their impact on growth improvement of tomato. Therefore, in the current study, effectiveness of mycorrhizal fungus (*Glomus mosseae*) and neemex alone and in combination was assessed against *M. incognita* using tomato under greenhouse conditions.

MATERIALS AND METHODS

Tomato seeds were surface sterilized and rinsed thoroughly with distilled water. The seeds were placed on autoclaved filter papers soaked with sterile distilled water and incubated at 25°C for 24 h. Four seedlings were planted in each pot and were thinned to one per pot after the emergence of the second leaf. Mycorrhizal fungus (MF) was propagated on maize host plants in a growth chamber at 30°C/18°C with a 16 h/8 h light/dark regime and 50–75% relative humidity. The plants were harvested after 4 months of growth. Soils containing spores, external mycelium and roots of maize were used as inoculum. Soil and sand mixture (1 kg) and 200 g mycorrhizal inoculum and 5 g neemex alone and in combination were placed in each pot 20 days before inoculation of nematodes. The inoculum was placed about 2 cm under the soil surface. Untreated pots received an equivalent amount of soil to provide a similar soil microbial community (Calvet et al., 2001). *Meloidogyne incognita* was isolated from heavily infected tomato roots and was multiplied on susceptible tomato cultivar using single egg mass. The number of galls on the roots was recorded. Egg masses were stained in phloxine B for 20 min, rinsed in sterilized distilled water and then counted under a stereomicroscope (Hussey, 1973). The number of female nematodes within the roots was counted using a dissecting microscope and staining by the NaOCl-acid fuchsin technique (Bybd et al., 1983). Similarly, data regarding growth parameters were recorded as described by (Hussain et al., 2016).

All the data were subjected to Analysis of Variance by using MINITAB/STAT statistical analysis software and means were compared by DMRT (Minitab, 2014).

RESULTS AND DISCUSSION

Effect of mycorrhizal fungus and neemex against *M. incognita:* The maximum number of galls was recorded in *M. incognita* treatment (327) while minimum galls were observed in roots of plants treated with the combined application of MF and neemex (43) followed by application of MF (52) and neemex alone (103) at $P<0.05$. Lower number of egg masses and females were recorded in MF plus neemex application (80 and 127) followed by MF (37 and 77) and neemex alone (72 and 123) as compared to control (Table 1). Second stage juveniles (J2)/root system and J2/100 cm^3 soil were estimated to be lower in combined treatment of MF and neemex (2430, 349) followed by MF (2754, 399) and neemex alone (4933, 677) as compared to check (12148, 1727). Root weight was significantly higher in *M. incognita* inoculated plants (7.4 g) followed by MF and neemex together (6.98 g) and MF alone in the presence of *M. incognita* (6.5 g) as compared to other treatments at $P<0.05$ (Table 2).

Significantly higher root length was recorded in plants treated with MF in the absence of *M. incognita* (19.31 cm) as compared to control. Un-inoculated healthy, combined application of MF and neemex without *M. incognita*, MF alone and MF with neemex in the presence of *M. incognita* were statistically significant over control but non-significant with each other at $P<0.05$. Shoot weight and shoot length was significantly higher in mycorrhizal treated plants (21.10 g, 29.13 cm respectively) as compared to other treatments.

Table 1. Effect of mycorrhizal fungus and neemex against *M. incognita* infectivity parameters.

Treatment	No. of galls	No. of Egg masses	No. of Females	J2/ root system	J2/100 cm^3 soil
Healthy	0 E	0 E	0 E	00 E	0 E
M. incognita	327 A	302 A	359 A	12148 A	1727 A
MF + Healthy	0 E	0 E	0 E	0 E	0 E
Neemex + Healthy	0 E	0 E	0 E	0 E	0 E
MF + Neemex +Healthy	0 E	0 E	0 E	0 E	0 E
MF + *M. incognita*	52 C	37 C	77 C	2754 C	399 C
Neemex + *M. incognita*	103 B	72 B	123 B	4933 B	677 B
MF + Neemex + *M. incognita*	43 D	42 D	80 D	2430 D	349 D
LSD at P<0.05	4.1002	3.3910	3.8520	3.1885	3.0900

Means sharing similar letters are statistically non-significant at P<0.05.

Table 2. Effect of mycorrhizal fungus and neemex on plant growth parameters.

Treatment	Root weight (g)	Root length (cm)	Shoot weight (g)	Shoot length (cm)
Healthy	5.6 BC	18.1300 AB	15.0900 CD	24.7800 B
M. incognita	7.4 A	10.0200 D	8.3900 E	16.6300 C
MF + Healthy	6.2 C	19.31 A	21.1000 A	29.1300 A
Neemex + Healthy	4.2 BC	17.1000 BC	13.5700 D	19.9500 C
MF + Neemex + Healthy	6.98 AB	19.3400 AB	17.8700 BC	27.0400 AB
MF + *M. incognita*	6.5 AB	19.6700 AB	19.9100 AB	27.6000 AB
Neemex + *M. incognita*	4.5 C	15.7800 C	13.6900 D	19.0300 C
MF + Neemex + *M. incognita*	6.1 BC	19.5100 AB	17.7300 BC	26.4900 AB
LSD at P<0.05	2.2173	3.2381	3.1947	3.6775

Means sharing similar letters are statistically non-significant at P<0.05.

Application of MF prior to nematode inoculation suppressed the nematodes to a greater degree than the application of MF after the nematodes. This might be related to the time interval necessary for the establishment of the mycorrhiza in the root cortex. It is well known that mycorrhiza establish in the root cortex in about 15 to 20 days in tomato (Sitaramaiah and Singh, 1978) and cotton (Saleh and Sikora, 1984). The presence of mycorrhiza in the host can reduce attraction to roots and juvenile penetration and retard nematode development after penetration (Sikora, 1992). Mycorrhizal spore population and mycorrhizal colonization were found to be lower when nematodes were inoculated before MF application. This agrees with previous observations in rough lemon seedlings infected with *Radopholus similis* and *G. etunicatum* in which vesicle formation and mycelial growth were lower in nematode infected roots. Also, reduced spore production in the presence of *M. incognita* may indicate competition for nutrients. Delaying application of MF after the nematode inoculation resulted in increased root galling and nematode population in the soil and suppression of spore population and colonization by MF. Changes in MF colonization and spore population in the presence of nematodes have been observed previously (Castillo et al., 2006; Waceke et al., 2001) and were attributed mainly to competition between MF and *Meloidogyne* spp. for feeding sites and carbon substrates from host photosynthesis (Hol and Cook, 2005). After *Meloidogyne* spp. invade the vascular cylinder, the root tissue around developing females usually proliferates to form knots or galls, which disrupt vessels and thus reduce the transport of water and nutrients through the altered roots. This may interfere with the translocation of metabolites

required by mycorrhizal fungi. The disease syndrome initiated by root-knot nematodes very often includes the invasion of affected root tissue by secondary pathogens, which cause decay of root tissues including the cortical tissue colonized by MF. Mycorrhizal development and growth of mycorrhizal and non-mycorrhizal plants were reported to be reduced in the presence of *M. arenaria* in grapes (Atilano et al., 1981). It is therefore, concluded from the present study that mycorrhizal fungus *Glomus mosseae* and neemex alone and in combination has the potential to reduce root-knot infestation and enhance growth of tomato.

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